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Rate of Gene Transfer From Mitochondria to Nucleus: Effects of Cytoplasmic Inheritance System and Intensity of Intracellular Competition

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ABSTRACT

Endosymbiotic theory states that mitochondria originated as bacterial intracellular symbionts, the size of the mitochondrial genome gradually reducing over a long period owing to, among other things, gene transfer from the mitochondria to the nucleus. Such gene transfer was observed in more genes in animals than in plants, implying a higher transfer rate of animals. The evolution of gene transfer may have been affected by an intensity of intracellular competition among organelle strains and the organelle inheritance system of the organism concerned. This article reveals a relationship between those factors and the gene transfer rate from organelle to nuclear genomes, using a mathematical model. Mutant mitochondria that lose a certain gene by deletion are considered to replicate more rapidly than normal ones, resulting in an advantage in intracellular competition. If the competition is intense, heteroplasmic individuals possessing both types of mitochondria change to homoplasmic individuals including mutant mitochondria only, with high probability. According to the mathematical model, it was revealed that the rate of gene transfer from mitochondria to the nucleus can be affected by three factors, the intensity of intracellular competition, the probability of paternal organelle transmission, and the effective population size. The gene transfer rate tends to increase with decreasing intracellular competition, increasing paternal organelle transmission, and decreasing effective population size. Intense intracellular competition tends to suppress gene transfer because it is likely to exclude mutant mitochondria that lose the essential gene due to the production of lethal individuals.

ENDOSYMBIOTIC theory states that mitochondria originated as bacterial intracellular symbionts, their genome size having become gradually reduced over a long period of symbiosis. In animals, mitochondrial genome sizes are quite small (16–20 kb), with only 37 genes in general lacking introns, in which the coding regions constitute >90% of their size (Gray 1989, 1992; Boore 1999). On the other hand, the genome size of plant mtDNA varies among species (160–2000 kb in angiosperms), with coding regions constituting 10% of the total mitochondrial genome and with many introns present (Gray 1989, 1992; Brown 1999). For example, in Arabidopsis, mtDNA contains 57 genes with 366,924 nucleotides (Unseld *et al.* 1997). Either way, these mitochondrial genome sizes are ~100-fold smaller than those of free-living bacteria (4000–6000 kb) (Selosse *et al.* 2001).

One process resulting in reduced mtDNA size is gene transfer from the organelle to the nucleus (Thorsness and Weber 1996). In higher organisms, gene transfer has been implied by the various locations of certain genes coding mitochondrial proteins among different organisms. For example, the α-subunit of F_1 ATPase exists in mitochondrial DNA in some eukaryotes but in nuclear DNA in others (Gray 1992), and the ribosomal protein gene *rps10* exists in the mitochondrial genome in some angiosperm species, but in the nuclear genome in others (Wischmann and Schuster 1995; Adams *et al.* 2000). It has also been reported that the respiratory gene *cox2*, which is normally present in mitochondria, is variably involved in the nuclear genome in legume species. Some legume species possess the gene in both the mitochondrial and nuclear genomes, some in the mitochondrial genome only, and others in the nuclear genome only (Adams *et al.* 1999).

A number of hypotheses have been proposed to explain why and how gene transfer from mitochondria to the nucleus took place. If a mitochondrial genome lacks recombinations, its genetic information may be lost according to Muller's ratchet. Consequently, once a mitochondrial gene is copied to a nuclear genome, the original mitochondrion-based gene degenerates more rapidly, resulting in the gene persisting only in the nucleus (Blanchard and Lynch 2000; Selosse *et al.* 2001). Nevertheless, the efficacy of Muller's ratchet may depend upon mutation rates. When the mutation rate differs notably between genomes, the copy in the genome with the higher mutation rate is considered to

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degenerate more rapidly, even under Muller's ratchet. In plants, the rate of nuclear mutation is orders of magnitude greater than the mitochondrial mutation rate (Wolfe *et al.* 1987), resulting in a low expectation of any gene transfer. Nevertheless, in reality, many genes have been lost from mitochondrial genomes, the nuclear copies instead being active in these species. Such a strong selective force for gene transfer cannot be explained by Muller's ratchet only (Blanchard and Lynch 2000).

Another hypothesis of gene transfer is that compactness of organelle genomes is advantageous in intracellular competition (Blanchard and Lynch 2000; Rand 2001; Selosse *et al.* 2001). If a mtDNA deletion mutant replicates faster than the wild-type full-length mtDNA, it will become more common in the cytoplasm. However, it can completely replace the wild-type mtDNA only if selection at the level of the cell allows the deletion mutant to persist without the functions encoded by the deleted region. On the basis of this concept, Albert *et al.* (1996) constructed a mathematical model of mitochondrial genome dynamics. They considered a three-level selection process consisting of intermolecular, intermitochondrial, and intercellular selection. The intermolecular selection was assumed to favor mitochondria with rapid replication, although both intermitochondrial and intercellular selection work against mitochondria lacking sufficient genetic information. There is no direct evidence for the intracellular selection for the rapid replication of mitochondria, although it has been suggested by the dynamics of yeast mitochondria involving good markers (respiration-deficient mutants, or *petites*). When heteroplasmic zygotes are produced by mating yeast strains that differ in one or more mitochondrial alleles, the majority of diploid progeny are homoplasmic after no more than 20 cell generations. In this case, the replication rate is considered to be one of several important factors causing homoplasmy (Birky 2001).

On the other hand, the current condition of transfer is also known to differ between plants and animals, having already been completed in the latter, although still continuing in plants (Brennicke *et al.* 1993). This implies that the gene transfer proceeded more rapidly in animals than in plants. The differing past gene transfer rates may be influenced by mutation rates involving gene insertions onto the nuclear genome and gene deletions from the mitochondria. Wolfe *et al.* (1987) reported that the synonymous substitution rate of nuclear DNA did not differ significantly between animals and plants, although the rate for mitochondrial DNA in the former is at least $\geq$100 times greater than that in the latter. This suggests that the mutation rate of mtDNA may result in a difference in gene transfer rate between them. Nevertheless, the effect of mutation rate of mtDNA on the gene transfer process is not clear. Accordingly, to find an answer to this issue, the process of gene transfer should be analyzed theoretically.

I consider that the gene transfer rate is affected by both an intensity of intracellular competition among organelle strains and an organelle inheritance system, the latter determining the probability of occurrence of intracellular competition. In many sexually reproducing organisms, organelle genomes are generally inherited by offspring from a single parent. Organelle inheritance can be originally a biparental system, with a uniparental system evolving subsequently by the suppression of inheritance from one parent (Hoekstra 1987; Hurst 1990, 1996; Hurst and Hamilton 1992). The intermediate stage of the evolutionary process of the organelle inheritance system can be considered as a state between the uniparental and biparental systems. If the evolutionary stage of the organelle inheritance system varied among organisms, the selective intensity for small organelle size may have differed among them, resulting in varying rates of gene transfer from organelle to nuclear genomes. The relationship among intensity of intracellular competition, organelle inheritance system, and gene transfer is considered below, using a mathematical model.

MODEL

Dynamics and equilibria: The model considers the transfer of a certain gene from the mitochondria to the nucleus in a diploid organism. The focused gene is essential for mitochondrial activities, which initially exist in the mitochondria, but not in the nuclear genome. In the model, mitochondrial and nuclear genomes are denoted as M and A, respectively. A genome including the focused functional gene is represented by a superscript +, with that excluding the gene being indicated by a superscript −. According to these definitions, in the initial stage an individual genotype is represented by A^-A^-/M^+, implying that the focused gene is coded by mitochondria only. On the other hand, after the transfer of the gene from mitochondrial to nuclear genomes has taken place, the gene is included only in the nuclear genome, where the population comprises individuals possessing genotype A^+A^+/M^-. The evolutionary process from A^-A^-/M^+ to A^+A^+/M^- possibly includes two steps. The first step involves insertion and activation of the gene on the nuclear genome, resulting in an A^+ allele, while the second step is deletion of the gene on the mitochondria genomes, bearing M^- type of mitochondria.

Since these two steps could proceed in parallel, there are intermediate stages between the initial and final stages, where A^- and/or A^+ nuclear genomes and M^+ and/or M^- mitochondrial genomes exist simultaneously in the population. In addition to the population level coexistence of M^+ and M^- mitochondria, they possibly also coexist within a single individual, *i.e.*, heteroplasmy. An individual that possesses M^+ and M^- mitochondria simultaneously is represented by $M^\pm$. M^--type mitochondria can be considered to arise from

TABLE 1

Offspring genotypes with respect to nuclear and mitochondrial genomes

	Male								
Female	A^-A^-/M^+	$A^-A^-/M^\pm$	A^-A^-/M^-	A^+A^-/M^+	$A^+A^-/M^\pm$	A^+A^-/M^-	A^+A^+/M^+	$A^+A^+/M^\pm$	A^+A^+/M^-
A^-A^-/M^+	A^-A^-/M^+	A^-A^-/M^+ $\underline{A^-A^-/M^\pm}$	—	A^-A^-/M^+ A^+A^-/M^+	A^-A^-/M^+ A^+A^-/M^+ $A^-A^-/M^\pm$ $\underline{A^+A^-/M^\pm}$	A^-A^-/M^+ A^+A^-/M^+ $A^-A^-/M^\pm$ $\underline{A^+A^-/M^\pm}$	A^+A^-/M^+	A^+A^-/M^+ $\underline{A^+A^-/M^\pm}$	A^+A^-/M^+ $\underline{A^+A^-/M^\pm}$
$A^-A^-/M^\pm$	$A^-A^-/M^\pm$	$A^-A^-/M^\pm$	—	$A^-A^-/M^\pm$ $A^+A^-/M^\pm$	$A^-A^-/M^\pm$ $A^+A^-/M^\pm$	$A^-A^-/M^\pm$ $A^+A^-/M^\pm$	$A^+A^-/M^\pm$	$A^+A^-/M^\pm$	$A^+A^-/M^\pm$
A^-A^-/M^-	—	—	—	—	—	—	—	—	—
A^+A^-/M^+	A^-A^-/M^+ A^+A^-/M^+	A^-A^-/M^+ A^+A^-/M^+ $A^-A^-/M^\pm$ $\underline{A^+A^-/M^\pm}$	—	A^-A^-/M^+ A^+A^-/M^+ A^+A^+/M^+	A^-A^-/M^+ A^+A^-/M^+ A^+A^+/M^+ $A^-A^-/M^\pm$ $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	A^-A^-/M^+ A^+A^-/M^+ A^+A^+/M^+ $A^-A^-/M^\pm$ $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	A^+A^-/M^+ A^+A^+/M^+	A^+A^-/M^+ A^+A^+/M^+ $A^+A^-/M^\pm$ $\underline{A^+A^+/M^\pm}$	A^+A^-/M^+ A^+A^+/M^+ $A^+A^-/M^\pm$ $\underline{A^+A^+/M^\pm}$
$A^+A^-/M^\pm$	$A^-A^-/M^\pm$ $A^+A^-/M^\pm$	$A^-A^-/M^\pm$ $A^+A^-/M^\pm$	—	$A^-A^-/M^\pm$ $A^+A^-/M^\pm$ $A^+A^+/M^\pm$	$A^-A^-/M^\pm$ $A^+A^-/M^\pm$ $A^+A^+/M^\pm$	$A^-A^-/M^\pm$ $A^+A^-/M^\pm$ $A^+A^+/M^\pm$	$A^+A^-/M^\pm$ $A^+A^+/M^\pm$	$A^+A^-/M^\pm$ $A^+A^+/M^\pm$	$A^+A^-/M^\pm$ $A^+A^+/M^\pm$
A^+A^-/M^-	(A^-A^-/M^-) A^+A^-/M^- $\underline{A^-A^-/M^\pm}$ $\underline{A^+A^-/M^\pm}$	(A^-A^-/M^-) A^+A^-/M^- $\underline{A^-A^-/M^\pm}$ $\underline{A^+A^-/M^\pm}$	—	(A^-A^-/M^-) A^+A^-/M^- A^+A^+/M^- $A^-A^-/M^\pm$ $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	(A^-A^-/M^-) A^+A^-/M^- A^+A^+/M^- $A^-A^-/M^\pm$ $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	(A^-A^-/M^-) A^+A^-/M^- A^+A^+/M^-	A^+A^-/M^- A^+A^+/M^- $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	A^+A^-/M^- A^+A^+/M^- $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	A^+A^-/M^- A^+A^+/M^-
A^+A^+/M^+	A^+A^-/M^+	A^+A^-/M^+ $\underline{A^+A^-/M^\pm}$	—	$\underline{A^+A^-/M^+}$ A^+A^+/M^+	$\underline{A^+A^-/M^+}$ A^+A^+/M^+ $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	A^+A^-/M^+ A^+A^+/M^+ $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	A^+A^+/M^+	A^+A^+/M^+ $\underline{A^+A^+/M^\pm}$	A^+A^+/M^+ $\underline{A^+A^+/M^\pm}$
$A^+A^+/M^\pm$	$A^+A^-/M^\pm$	$A^+A^-/M^\pm$	—	$A^+A^-/M^\pm$ $A^+A^+/M^\pm$	$\underline{A^+A^-/M^\pm}$ $A^+A^+/M^\pm$	$\underline{A^+A^-/M^\pm}$ $A^+A^+/M^\pm$	$A^+A^+/M^\pm$	$A^+A^+/M^\pm$	$A^+A^+/M^\pm$
A^+A^+/M^-	A^+A^-/M^- $\underline{A^+A^-/M^\pm}$	A^+A^-/M^- $\underline{A^+A^-/M^\pm}$	—	A^+A^-/M^- A^+A^+/M^- $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	$\underline{A^+A^-/M^-}$ A^+A^+/M^- $\underline{A^+A^-/M^\pm}$ $\underline{A^+A^+/M^\pm}$	A^+A^-/M^- A^+A^+/M^-	A^+A^+/M^- $\underline{A^+A^+/M^\pm}$	A^+A^+/M^- $\underline{A^+A^+/M^\pm}$	A^+A^+/M^-

Parentheses indicate lethal genotypes owing to a lack of the essential gene. Underlines indicate offspring that are reproduced via biparental organelle inheritance.

the M^+ type by gene deletion. Therefore, the genome size of the former would be smaller than that of the latter. Under conditions of intracellular competition between these mitochondrial strains, compactness of genome size can be advantageous due to the high replication rate (Blanchard and Lynch 2000; Selosse *et al.* 2001). Consequently, it is assumed that $M^\pm$ individuals change to M^- individuals in each generation with a certain probability, α, the latter representing the intensity of intracellular competition. Accordingly to these considerations, the dynamics include nine genotypes, A^-A^-/M^+, $A^-A^-/M^\pm$, A^-A^-/M^-, A^+A^-/M^+, $A^+A^-/M^\pm$, A^+A^-/M^-, A^+A^+/M^+, $A^+A^+/M^\pm$, and A^+A^+/M^-. The third genotype, A^-A^-/M^-, cannot exist substantially because the individual lacks the essential gene.

Next, an organelle inheritance pattern is considered. Recently, many organisms have been found to possess a uniparental cytoplasmic inheritance system, although the system can be considered to have been biparental in their ancestors. The model assumes that organelle inheritance is between the biparental and uniparental systems, in which one parent (maternal) always transfers the organelle to offspring, the other parent (paternal) often transferring it at a certain level of probability, represented by p. When $p \neq 0$, the mating consequences can be summarized as in Table 1. In the model, a hermaphrodite organism is assumed, genotypic frequencies of A^-A^-/M^+, $A^-A^-/M^\pm$, A^-A^-/M^-, A^+A^-/M^+, $A^+A^-/M^\pm$, A^+A^-/M^-, A^+A^+/M^+, $A^+A^+/M^\pm$, and A^+A^+/M^- being denoted by x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, and x_9, respectively. In the presented analysis, frequency dynamics are considered to involve two steps. The first step is mating among individuals, with the second being a genotypic change from $M^\pm$ to M^- as a result of the intracellular competition. On the basis of the mating

consequence shown in Table 1, the frequency dynamics in the first step can be formulated by

$$x_1' = (x_1 + \tfrac{1}{2}x_4)\{x_1 + (1-p)x_2 + \tfrac{1}{2}x_4 + \tfrac{1}{2}(1-p)(x_5 + x_6)\}, \quad (1a)$$

$$x_2' = x_1\{px_2 + \tfrac{1}{2}p(x_5 + x_6)\} + x_2\{x_1 + x_2 + \tfrac{1}{2}(x_4 + x_5 + x_6)\} + \tfrac{1}{2}px_4\{x_2 + \tfrac{1}{2}(x_5 + x_6)\} + \tfrac{1}{2}x_5\{x_1 + x_2 + \tfrac{1}{2}(x_4 + x_5 + x_6)\} + \tfrac{1}{2}x_6\{x_1 + x_2 + \tfrac{1}{2}p(x_4 + x_5)\}, \quad (1b)$$

$$x_3' = 0, \quad (1c)$$

$$x_4' = x_1\{\tfrac{1}{2}x_4 + \tfrac{1}{2}(1-p)(x_5 + x_6) + x_7 + (1-p)(x_8 + x_9)\} + \tfrac{1}{2}x_4\{x_1 + (1-p)x_2 + x_4 + (1-p)(x_5 + x_6) + x_7 + (1-p)(x_8 + x_9)\} + x_7\{x_1 + (1-p)x_2 + \tfrac{1}{2}x_4 + \tfrac{1}{2}(1-p)(x_5 + x_6)\}, \quad (1d)$$

$$x_5' = px_1\{\tfrac{1}{2}(x_5 + x_6) + x_8 + x_9\} + x_2\{\tfrac{1}{2}(x_4 + x_5 + x_6) + x_7 + x_8 + x_9\} + \tfrac{1}{2}px_4(x_2 + x_5 + x_6 + x_8 + x_9) + \tfrac{1}{2}x_5(x_1 + x_2 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9) + \tfrac{1}{2}px_6(x_1 + x_2 + x_4 + x_5 + x_7 + x_8) + px_7\{x_2 + \tfrac{1}{2}(x_5 + x_6)\} + x_8\{x_1 + x_2 + \tfrac{1}{2}(x_4 + x_5 + x_6)\} + px_9\{x_1 + x_2 + \tfrac{1}{2}(x_4 + x_5)\}, \quad (1e)$$

$$x_6' = \tfrac{1}{2}x_6\{(1-p)(x_1 + x_2 + x_4 + x_5 + x_7 + x_8) + (x_6 + x_9)\} + x_9\{(1-p)(x_1 + x_2) + \tfrac{1}{2}(1-p)(x_4 + x_5) + \tfrac{1}{2}x_6\}, \quad (1f)$$

$$x_7' = (\tfrac{1}{2}x_4 + x_7)\{\tfrac{1}{2}x_4 + \tfrac{1}{2}(1-p)(x_5 + x_6) + x_7 + (1-p)(x_8 + x_9)\}, \quad (1g)$$

$$x_8' = \tfrac{1}{2}px_4\{\tfrac{1}{2}(x_5 + x_6) + x_8 + x_9\} + \tfrac{1}{2}x_5\{\tfrac{1}{2}(x_4 + x_5 + x_6) + x_7 + x_8 + x_9\} + \tfrac{1}{2}px_6\{\tfrac{1}{2}(x_4 + x_5) + x_7 + x_8\} + px_7\{\tfrac{1}{2}(x_5 + x_6) + x_8 + x_9\} + x_8\{\tfrac{1}{2}(x_4 + x_5 + x_6) + x_7 + x_8 + x_9\} + px_9\{\tfrac{1}{2}(x_4 + x_5) + x_7 + x_8\}, \quad (1h)$$

$$x_9' = (\tfrac{1}{2}x_6 + x_9)\{\tfrac{1}{2}(1-p)(x_4 + x_5) + \tfrac{1}{2}x_6 + (1-p)(x_7 + x_8) + x_9\}, \quad (1i)$$

where x_i' indicate relative frequencies immediately after the mating step. If a dioecious organism is assumed with sex ratio s, its frequency dynamics can be formulated by simply multiplying $s(1-s)$ to Equations 1a–1i. However, such a modification does not alter the characteristics or behavior of the system. In the second step, $M^{\pm}$ individuals change to M^- with probability α because M^- mitochondria are superior in the intracellular competition to some degree. The dynamics are

$$x_1'' = x_1'/W, \quad (1j)$$

$$x_2'' = (1-\alpha)x_2'/W, \quad (1k)$$

$$x_3'' = 0, \quad (1l)$$

$$x_4'' = x_4'/W, \quad (1m)$$

$$x_5'' = (1-\alpha)x_5'/W, \quad (1n)$$

$$x_6'' = (x_6' + \alpha x_5')/W, \quad (1o)$$

$$x_7'' = x_7'/W, \quad (1p)$$

$$x_8'' = (1-\alpha)x_8'/W, \quad (1q)$$

$$x_9'' = (x_9' + \alpha x_8')/W, \quad (1r)$$

where x_i'' represent frequencies in the next generation and W is average fitness, $W = x_1' + (1-\alpha)x_2' + x_4' + x_5' + x_6' + x_7' + x_8' + x_9'$.

According to Equation 1, the system has three equilibria. One equilibrium is $(x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, x_9) = ((1-r)^2, 0, 0, 2r(1-r), 0, 0, r^2, 0, 0)$, in which r represents the gene frequency of A^+ alleles in the population. This equilibrium is a set of points on a curve with varying r-value, which is feasible for any r-values $(0 \le r \le 1)$. In this equilibrium, the population comprises both A^- and A^+ alleles, although M^+ mitochondria dominate, thereby excluding M^-. According to the linearization analysis, one eigenvalue around this equilibrium is always 1, while other eigenvalues cannot be derived explicitly. Only when α is 1 (*i.e.*, heteroplasmy absent), the stability condition is simple; all eigenvalues around this equilibrium are between -1 and 1 if

$$r \le \frac{1-\sqrt{p}}{1+p} = r^*. \quad (2)$$

When this condition is satisfied, the equilibrium is neutrally stable (because one eigenvalue is always 1), where M^- mitochondria cannot increase in the population although frequency of the A^+ allele (r) can fluctuate by genetic drift. In condition (2), r^* represents a critical frequency of the A^+ alleles, below which an invasion of M^- mitochondria is prevented. The general stabilities of this equilibrium for various α-values are analyzed numerically, searching a critical value of r above which an eigenvalue around the equilibrium exceeds 1. Figure 1a indicates the stability condition, indicating that the stability depends upon the probability of paternal organelle transmission (p), intensity of intracellular competition (α), and gene frequency of the A^+ allele in the population (r). The equilibrium is neutrally stable in a region below the solid surface (r^*) (because one eigenvalue is always 1), but unstable above. In addition, it is analytically indicated that in Figure 1a an intersection of the solid surface (r^*) and the $\alpha - p$ plane (*i.e.*, $r = 0$) is represented by $\alpha/\{p(1-\alpha)\} = 1$.

The second equilibrium of the system is $(x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, x_9) = (0, 0, 0, 0, 0, 0, 0, 1-\alpha/\{p(1-\alpha)\}, \alpha/\{p(1-\alpha)\})$, at which the population involves $A^+A^+/M^{\pm}$ and A^+A^+/M^- individuals. The equilibrium is both feasible and stable only when $\alpha/\{p(1-\alpha)\} \le 1$ according to the stability analysis. The third equilibrium is $(x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, x_9) = (0, 0, 0, 0, 0, 0, 0, 0, 0, 1)$, implying that the population comprises only A^+A^+/M^- individuals. This equilibrium always exists, being always neutrally stable due to the maximum eigenvalue around the state being 1. When the first equilibrium comprising M^+ mitochondria only is unstable (see also Figure 1a), M^- can increase in the population, reaching either the second or the third equilibrium. In such cases, both the second and the third equilibria are bistable when $\alpha/\{p(1-\alpha)\} \le 1$, although the third equilibrium is a single stable point when $\alpha/\{p(1-\alpha)\} > 1$ (see frame boundary in Figure 1a). According to simulations, under the bistable situation the state tends to converge into the second equilibrium, resulting in the coexistence of $A^+A^+/M^{\pm}$ and A^+A^+/M^-. Figure 1b illustrates the frequency of A^+A^+/M^- genotypes that is ultimately achieved when the first equilibrium is unstable.

Consequently, the equilibria can be summarized as follows. The initial state A^-A^-/M^+ is neutrally stable if

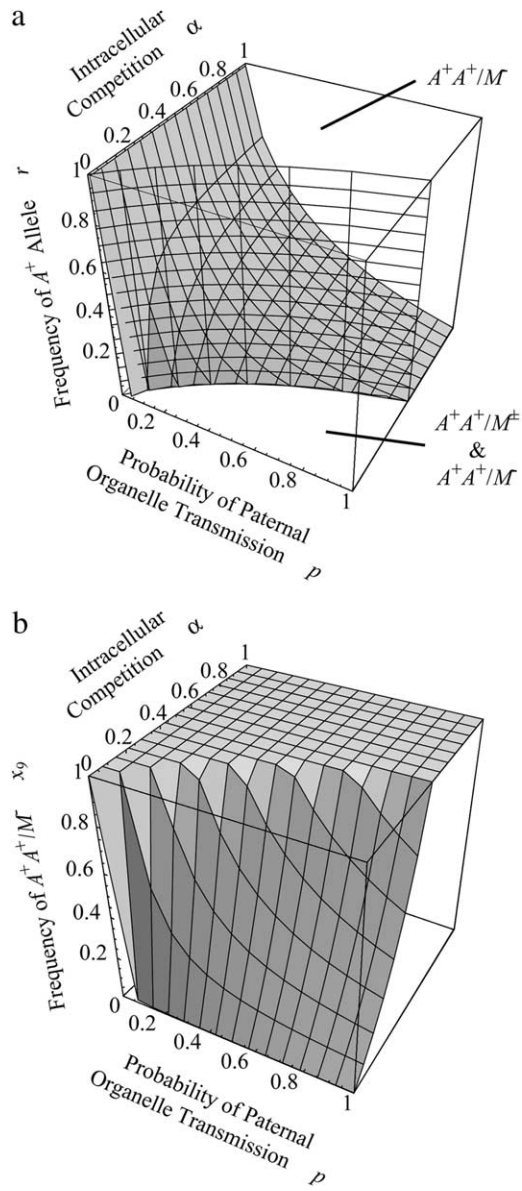

FIGURE 1.—(a) Stabilities of equilibria with various probabilities of paternal organelle transmission (p) and intensity of intracellular competition (α). When a coexistence of A^-A^-/M^+, A^+A^-/M^+, and A^+A^+/M^+ is unstable (above the solid surface), two cases exist: monostableness of A^+A^+/M^- and bistableness of dominance of A^+A^+/M^- and coexistence of $A^+A^+/M^\pm$ and A^+A^+/M^-. These two regions are separated by $\alpha/(1-\alpha) = p$ (frame surface). (b) Ultimate frequency of A^+A^+/M^- after an increment of M^- mitochondria. Under the bistable case, the dynamics are likely to converge to a coexistence equilibrium.

intracellular competition is relatively intense [*i.e.*, $\alpha/\{p(1-\alpha)\}>1$], where M^- mitochondria cannot invade the population, but frequency of the A^+ allele changes through genetic drift. If the frequency of the A^+ allele exceeds a critical value (r^*), M^- mitochondria begin to increase, the population finally reaching the A^+A^+/M^- state. On the other hand, if intracellular competition is relatively weak [*i.e.*, $\alpha/\{p(1-\alpha)\} \leq 1$], the initial state A^-A^-/M^+ is unstable independently of the frequency of the A^+ allele (in this case, r^* can be regarded as 0). In such cases, both the A^+ allele and M^- mitochondria increase, resulting in coexistence of $A^+A^+/M^\pm$ and A^+A^+/M^- genotypes.

When the frequency of the A^+ allele reaches the critical value ($r = r^*$), M^- mitochondria start to increase, ultimately reaching the steady frequency (either the second or the third equilibrium, see Figure 1, a and b). A period for which M^- increases until attaining a steady state is estimated by using a computer simulation based on the dynamics of Equation 1. Figure 2 illustrates the generation in which the frequency of A^+A^+/M^--type individuals increases to 99.9% of the steady state (see also Figure 1b) after the frequency of A^+ alleles reaches the critical value, which is plotted against both probabilities of paternal organelle transmission (p) and intensity of intracellular competition (α). In the simulation, the initial frequency of the A^+ allele (r) is set to r^* when $r^* \geq 10^{-6}$, although it is set to 10^{-6} when $r^* < 10^{-6}$. This is because the increment of M^- mitochondria is too slow to determine the generation by simulations if A^+ alleles are almost absent. Especially when the A^+ allele does not exist in the population at all, M^- mitochondria never increase. On the other hand, a single $M^\pm$ mutant individual initially occurs in the population, of which frequency depends on the population size. To analyze the effect of population size on evolutionary process, two types of initial frequency of $M^\pm$ genotype are examined, which are 10^{-6} and 10^{-3} in Figure 2, a and b, respectively. In the calculation, it is assumed that M^- mitochondria begin to increase immediately after the frequency of A^+ nuclear genes reaches r^*. For such an assumption, it is necessary that M^- mutant mitochondria persist with low frequency in the population by selection-mutation balance, even when $r < r^*$ (see DISCUSSION). Comparing Figure 2a and 2b, as the initial frequency of $M^\pm$ individuals becomes larger, the generations of increments of M^- individuals become fewer, especially in the high-α region.

Waiting time for increments of A^+ frequency from 0 to r^*: The above analysis shows that once the frequency of A^+ alleles reaches r^*, M^- mitochondria can invade the M^+ population, owing to the selective force for small genome size. To understand the overall process of gene transfer, the mechanism by which A^+ alleles increase to r^* in the M^+ population should be considered. During the process, the A^+ alleles are not exposed to any selective force because all mitochondria have the concerned gene, M^+. In this case, the gene frequency of A^+ alleles changes from generation to generation only by random genetic drift. The period, for which A^+ alleles increase to r^* in the M^+ population, can be separated into two phases. The first phase is a period waiting for the occurrence of a single A^+ mutant that ultimately increases to r^* in frequency without reaching 0, which is defined by τ_1. The second phase is a period

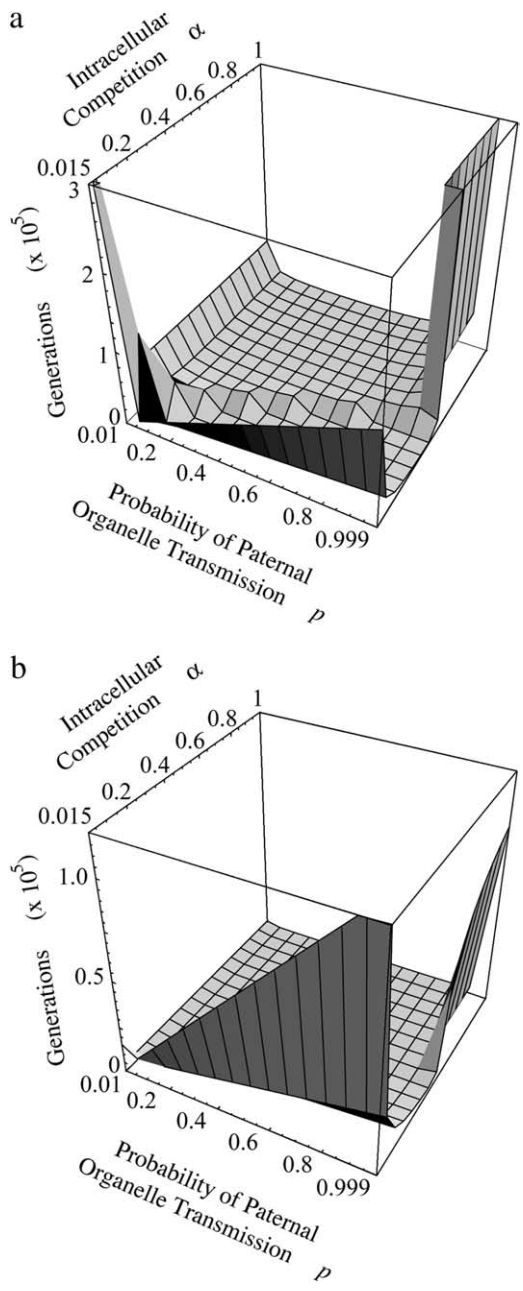

FIGURE 2.—Expected generations for A^+A^+/M^- individuals increasing upon 99.9% of the steady state (see also Figure 1b), after the frequency of A^+ alleles reaches r^*, plotted against various probabilities of paternal organelle transmission (p) and intensity of intracellular competition (α). Initial frequencies of $M^{\pm}$ individuals are (a) 10^{-6} and (b) 10^{-3}.

during which such a single A^+ mutant increases to r^* in the population, being τ_2.

Initially, the waiting time for the occurrence of a single A^+ mutant that ultimately increases to r^* is considered along a line similar to that in Kimura's neutral theory (KIMURA 1962, 1968, 1983). The rate of the occurrence of such an A^+ mutant is represented by the product of two factors, the probability of the A^+ mutant newly arising in each generation and the probability of such an allele increasing to r^* without reaching 0. When the population size (of diploid organisms) is N, the number of loci is $2N$. The mutation rate from A^- to A^+ and the probability of A^+ increasing until r^* without extinction are denoted by μ and u, respectively. The occurrence rate of a new A^+ mutant that ultimately increases until r^* before reaching 0 can be formulated by

$$k = 2N\mu u. \tag{3}$$

To determine the u-value, the increment process of the A^+ mutant is considered. In this process, bidirectional mutations can occur, one being a forward mutation from A^- to A^+ with rate μ, the other being a backward mutation from A^+ to A^- with rate ν. The backward mutation can result from various types of mutation, destroying the function of A^+. On the other hand, the establishment of a single forward mutation includes several steps. The concerned gene is first inserted into a chromosome by RNA-mediated transfer (WISCHMANN and SCHUSTER 1995), being subsequently functionalized by the acquisition of both an adjacent promoter sequence and a peptide sequence for targeting them back to the organelle. Consequently, the establishment of a single forward mutation must be a very rare event (*i.e.*, rate $\mu \ll 1$). Since the forward mutation is significantly more rare than the backward mutation ($\mu \ll \nu$), the former can be negligible in this random genetic drift process. Considering the backward mutation only, the probability of a single A^+ allele increasing from $1/2N$ to r^* without extinction can be formulated approximately by

$$u = \begin{cases} \dfrac{1-\{1-1/(2N)\}^{-4\nu N+1}}{1-(1-r^*)^{-4\nu N+1}} & \text{if } 1/2N < r^* \\ 1 & \text{if } 1/2N \geq r^* \end{cases} \tag{4}$$

(KARLIN and TAYLOR 1981). The average period within which a single new A^+ mutant that ultimately increases until r^* occurs is the inverse of rate k. From Equations 3 and 4, this can be represented by

$$\tau_1 = \frac{1}{k} = \begin{cases} \dfrac{1}{2N\mu}\dfrac{1-(1-r^*)^{-4\nu N+1}}{1-\{1-1/(2N)\}^{-4\nu N+1}} & \text{if } 1/2N < r^* \\ \dfrac{1}{2N\mu} & \text{if } 1/2N \geq r^*. \end{cases} \tag{5}$$

Consequently, the waiting time (and also the rate) for the occurrence of a single A^+ allele that ultimately increases until r^* depends upon the population size, being inconsistent with Kimura's neutral theory in which the average period is not dependent upon the latter. This inconsistency results from the combination of backward mutations with the genetic drift process in the analysis presented here, the neutral theory ignoring mutations in that process. Figure 3, a and b, illustrates τ_1-values against both the probability of paternal organelle transmission (p) and the intensity of intracellular

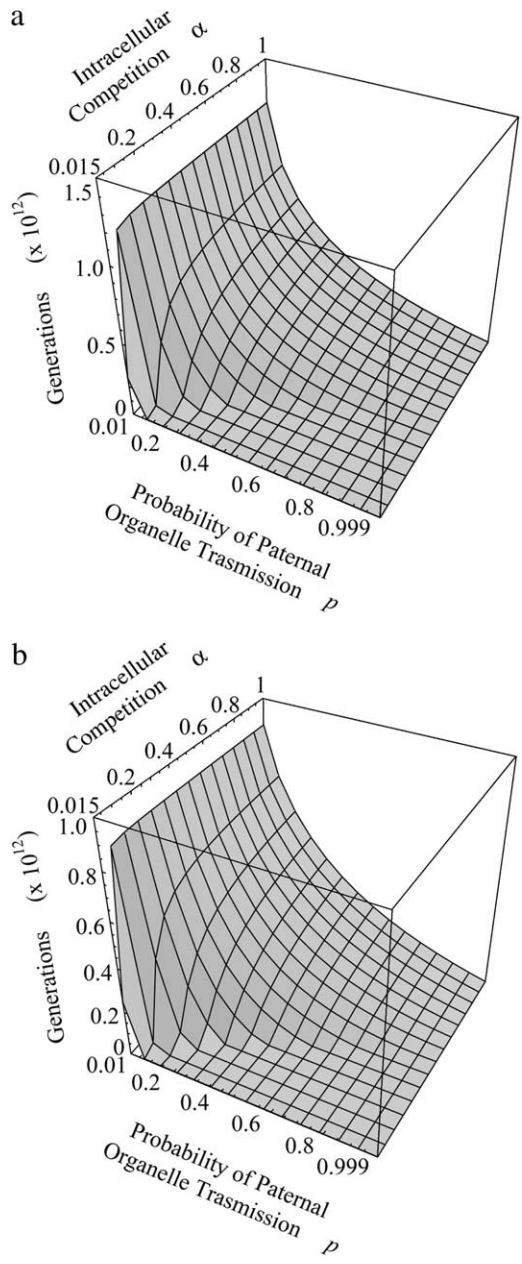

FIGURE 3.—Expected generations for occurrence of an autosomal mutant A^+ that can ultimately reach r^* without reaching $r = 0$, plotted against various probabilities of paternal organelle transmission (p) and intensity of intracellular competition (α). Rates of forward and backward autosomal mutations between A^- and A^+ alleles (μ and ν) are set at 10^{-12} and 10^{-7}, respectively. Effective population sizes (N) are (a) 10^6 and (b) 10^3.

competition (α), with $N = 10^6$ and 10^3, respectively. In Figure 3, a backward mutation rate, ν, from A^+ to A^- is set at 10^{-7} per loci per replication, being an autosomal loss of function of the concerned gene. This value was estimated from the rates of visible mutation (KIMURA 1983), some fraction of which may result from a loss of gene function. On the other hand, it is difficult to estimate a rate of forward mutations, μ, from A^- to A^+. Since forward mutations must be very rare compared with backward mutations ($\mu \ll \nu$), the value was set at 10^{-12}.

Next, the period during which the frequency of a single A^+ mutant increases from 0 to r^* without reaching 0 is considered. According to KARLIN and TAYLOR (1981), if $1/2N < r^*$ it can be formulated by

$$\tau_2 = \frac{4N}{(4\nu N-1)\{(1-r^*)^{-4\nu N+1}-1\}} \times \left[\frac{(1-r^*)^{-4\nu N+1}-\{1-1/(2N)\}^{-4\nu N+1}}{\{1-1/(2N)\}^{-4\nu N+1}-1} \times \int_0^{1/(2N)} \frac{\{(1-\xi)^{-4\nu N+1}-1\}^2}{\xi(1-\xi)^{-4\nu N+1}} d\xi + \int_{1/(2N)}^{r^*} \frac{\{(1-\xi)^{-4\nu N+1}-1\}\{(1-r^*)^{-4\nu N+1}-(1-\xi)^{-4\nu N+1}\}}{\xi(1-\xi)^{-4\nu N+1}} d\xi\right]. \quad (6)$$

On the other hand, $\tau_2 = 0$ when $1/2N \geq r^*$. Figure 4, a and b, illustrates τ_2-values against both the probability of paternal organelle transmission (p) and the intensity of intracellular competition (α), with $N = 10^6$ and 10^3, respectively. The mutation rates are set at the same levels as those in Figure 3.

Comparing Figures 2–4, under the parameters given, the total average period of transfer of a single gene is likely to be determined by τ_1, owing to the latter being much larger than any other period. Consequently, from Figure 3, the period for gene transfer decreases approximately with the probability of paternal organelle transmission (p) and increases with the intensity of intracellular competition (α) and population size (N). According to the analysis, the process of gene transfer can be considered to involve five evolutionary steps: (i) the population initially comprises individuals with the genotype A^-A^-/M^+ only; (ii) A^+A^-/M^+ and A^+A^+/M^+ individuals result from the occurrence of the autosomal mutant A^+ that is neutral for selection due to $r < r^*$; (iii) the frequency of A^+ alleles gradually increases until r^*, owing to genetic drift; (iv) when the frequency of A^+ alleles exceeds the critical value ($r > r^*$), mutant M^- mitochondria, having an advantage in intracellular competition, can spread through the population; and (v) gene transfer is completed either by fixation of the A^+A^+/M^- genotype or by coexistence of the $A^+A^+/M^\pm$ and A^+A^+/M^- genotypes. It is important that in these evolutionary processes, the average period is dependent upon the probability of paternal organelle transmission, p.

DISCUSSION

The analysis presented here demonstrates a notable effect of the intensity of intracellular competition on gene transfer rate from mitochondrial to nuclear DNA. At first glance, if M^- mitochondria replicate more rapidly than M^+ mitochondria [*i.e.*, a high intensity of intracellular competition (high α)], a state comprising

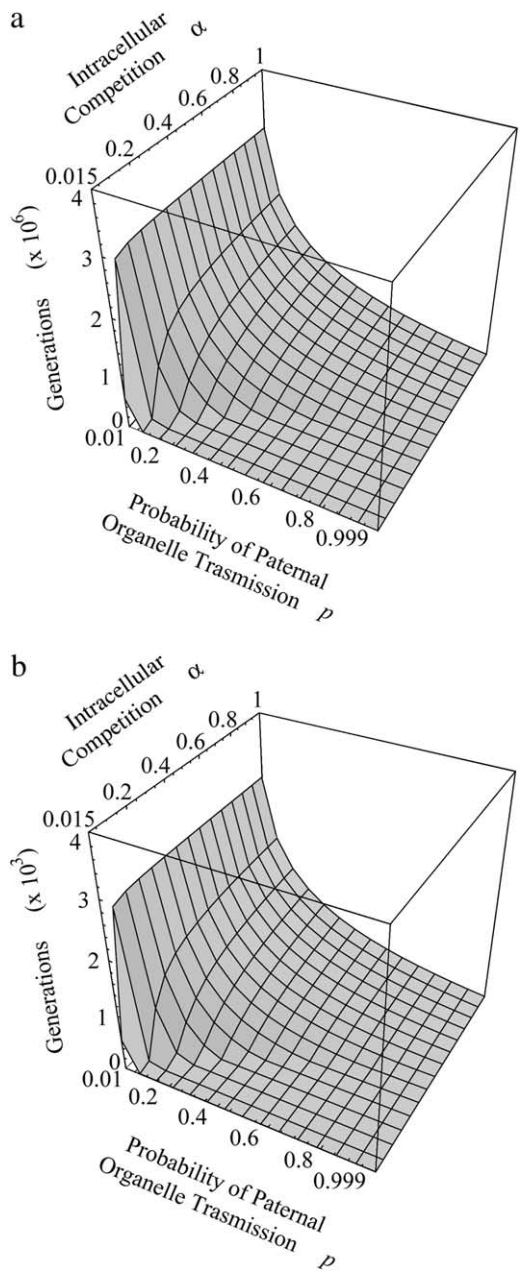

FIGURE 4.—Expected generations during which the frequency of mutant A^+ alleles increases from $1/2N$ to r^* without reaching 0, owing to random genetic drift. Generations are plotted against various probabilities of paternal organelle transmission (p) and intensity of intracellular competition (α). Rates of forward and backward autosomal mutations between A^- and A^+ alleles (μ and ν) are set at 10^{-12} and 10^{-7}, respectively. Effective population sizes (N) are (a) 10^6 and (b) 10^3.

A^-A^-/M^+ genotypes may likely lead to a state including A^+A^+/M^- only. However, Figures 3 and 4 show that the rate of gene transfer tends to be slower when intracellular competition is intense (high α). This tendency can be explained as follows. When the M^- mitochondria replicate more rapidly than M^+ mitochondria, heteroplasmic $M^\pm$ individuals tend to change to M^- with a high probability. Accordingly, $A^-A^-/M^\pm$ individuals are likely to die because the A^-A^-/M^- genotype is lethal, which significantly restricts an increment of M^-, especially in the population with a high frequency of A^- allele. Consequently, if intracellular competition is intense (high α), M^- can increase only when A^+ alleles achieve relatively high frequency (high r^*), this factor reducing the gene transfer rate. A weakness of intracellular competition facilitates gene transfer, although it also tends to result in the coexistence of $A^+A^+/M^\pm$ and A^+A^+/M^- genotypes (see Figure 1b). In such cases, the rapid replication of M^- mitochondria balances a decrement of M^- genotype that results from $M^\pm$ offspring production by mating between $M^\pm$ and M^- individuals due to biparental cytoplasmic inheritance. Such a coexisting situation may explain the locations of respiratory gene *cox2* in some legume species, this gene being active in both nuclear and mitochondrial genomes simultaneously in those species (ADAMS *et al.* 1999).

When heteroplasmy exists, genetic drift of mitochondria could allow rapid shifts in frequency of M^- genotype especially without intracellular competition. However, if intracellular competition is completely absent, M^- mitochondria cannot increase unless A^+ alleles fix in the population, because when A^+ and A^- alleles coexist, M^- genotype always suffers a disadvantage owing to the lethality of A^-A^-/M^- individuals. Accordingly, evolution of M^- is strongly restricted by the fixation of A^+ alleles. In addition to this, if intracellular competition does not exist, M^- genotype is difficult to increase, since M^- genotype tends to decrease due to production of $M^\pm$ individuals by mating between $M^\pm$ and M^- individuals (see also Figure 1, a and b). Consequently, the effect of genetic drift of mitochondria may not contribute to the increment of M^- genotype when intracellular competition is absent.

This analysis also showed that the rate of gene transfer from mitochondria to the nucleus depends upon the effective population size. The model demonstrated that the expected period of gene transfer increases with increasing population size (N) (see also Figures 3 and 4). Accordingly, if the effective population sizes of animals are smaller than those of plants, the gene transfer rate in the former is likely to be higher. This may suggest a small population size in a common ancestor of animals. The rate of gene transfer is also affected by the probability of paternal organelle transmission. The analysis indicated that the evolutionary period of gene transfer from mitochondrial to nuclear genomes tends to decrease with increasing paternal organelle inheritance probability (p). Since biparental organelle transmission increases opportunities for intracellular competition, a small organelle genome size is favored, resulting in the promotion of gene transfer. Accordingly, if plants had acquired a uniparental organelle inheritance system earlier than animals, or if those systems in plants work more exactly than those in

animals, gene transfer in plants may proceed more slowly than that in animals.

Maternal cytoplasmic inheritance has been considered to be an established feature in many angiosperms and all animals. Nevertheless, recent studies have suggested that biparental mitochondrial inheritance occurs in animals to some degree. Recently, analyses of human mitochondrial DNA have shown homoplasmies among populations, suggestive of recombinations of mtDNA (Awadalla *et al.* 1999; Eyre-Walker *et al.* 1999; Bromham *et al.* 2003), as such implying biparental mitochondrial inheritance. In mammalian fertilization, a sperm "midpiece," including 50–75 mitochondrial genomes, has been observed to enter the oocyte (Ankel-Simons and Cummins 1996). Studies using interspecific hybrids have also shown that paternal mitochondrial DNA can be transmitted to offspring in mice (Gyllensten *et al.* 1991) and Drosophila (Kondo *et al.* 1992). A subsequent study of mice, however, showed that although paternal mitochondrial DNA remains in the zygote until the neonate stage in interspecific crosses, it is eliminated from the oocyte in intraspecific crosses (Kaneda *et al.* 1995), indicating that biparental organelle transmission might be specific for interspecific hybrids only. Nevertheless, these studies have suggested, at least, that paternal mitochondrial transmission tends to be prevented by molecular mechanisms in animals, rather than by a physical mechanism.

On the other hand, many angiosperms have also adopted maternal cytoplasmic inheritance systems (Mogensen 1996). In contrast to animals, a physical mechanism preventing paternal cytoplasmic transmission may have evolved in some angiosperms. In barley (*Hordeum vulgare*), a sperm nucleus enters an egg cell at fertilization, although the cytoplasmic body of the sperm cell is left outside the egg (Mogensen 1996). In addition to this, leakage of male plastids has been reported for several angiosperm species, although no mitochondrial leakage has been reported, suggesting that angiosperms have a more effective mechanism for preventing paternal mitochondrial transmission than animals. These characteristic differences in mitochondrial inheritance between plants and animals may influence their differing gene transfer rates from mitochondrial to nuclear genomes. However, the present study cannot explain factors resulting in different levels of evolution of cytoplasmic inheritance systems. Such questions may be answered by considering the evolution of uniparental cytoplasmic inheritance systems (for a review, see Partridge and Hurst 1998).

This analysis predicted that despite the difference in waiting time, all mitochondrial genes may ultimately transfer from mitochondria to the nuclear genome, resulting in disappearance of mitochondria. Since such a prediction is unrealistic, any factors may possibly prevent complete transfer of genes from mitochondria to nucleus, thus maintaining mitochondrial genes. Allen (1993) supposed a hypothesis that maintenance of organelle genomes concerns "redox response regulation." Structural proteins that maintain redox balance in bioenergetic membranes must be synthesized when and where those are needed. This straightforward and potentially profound selective pressure maintains genomes in organelles over evolutionary time. Race *et al.* (1999) also stated that the recent data supported Allen's hypothesis. In addition to this, the present analysis showed that if cytoplasmic inheritance was completely uniparental ($p = 0$), mitochondria without the concerned gene cannot spread in the population, owing to the absence of intracellular competition even when the nuclear genome with the gene increases through genetic drift. It suggested that the establishment of uniparental cytoplasmic inheritance systems might prevent gene transfer events currently.

The gene transfer rate may be also influenced by the occurrence rate of M^- mitochondria from M^+ mitochondria. Nevertheless, this analysis assumed that M^- mutant mitochondria always persist in the population even when $r < r^*$; therefore, the $M^\pm$ and M^- genotypes can increase immediately after r exceeds r^* (see Figure 2). This assumption implies that M^- mutant mitochondria frequently occur in the population, despite their being excluded by negative selection under $r < r^*$. This assumption was based on the following consideration. If the mutation rate from M^+ to M^- is similar to that from A^+ to A^-, the rate may be $\sim 10^{-7}$ ($\cong \nu$) per loci per replication. Although the number of mtDNA copies in a single cell is not clearly understood (Brown 1999), the former is approximately some thousands. If an organism has 10^4 mtDNA copies per cell, and a germ cell experiences 10 instances of division in a single generation, an individual could have 10^{-2} ($=10^{-7} \times 10^4 \times 10$) mutations from M^+ to M^- per generation. If the population size is >100, some new $M^\pm$ individuals possibly occur in the population in every generation, even when selection works against M^- mitochondria. Accordingly, $M^\pm$ and M^- genotypes are assumed to increase immediately after r exceeds r^*. The variable mutation rate of mtDNA possibly affects this process to some extent, although its effect on the overall waiting time may not be significant because τ_1 is very large in comparison to the increment process of M^- mitochondria (see Figures 2 and 3). Accordingly, differences in the mutation rate of mtDNA may not be a primary factor responsible for the differing gene transfer rates among plants and animals.

In this analysis, the A^+ nuclear allele is considered completely neutral for selection, although A^+ might affect individual fitness and thus influence the evolutionary process of gene transfer from mitochondria to nucleus. If the effect of A^+ on fitness is significant (*i.e.*, strong relative to genetic drift), the result can be clearly predicted. When the A^+ allele is explicitly disadvantageous, it is rapidly eliminated from the population

comprising M^+ mitochondria only, resulting in no gene transfer from mtDNA to nuclear genome. On the other hand, when the A^+ allele is explicitly advantageous, it fixes in the population soon after its occurrence. In such a case, the waiting time until the frequency of the A^+ alleles reaches the critical value r^* may be almost negligible. In contrast, if the effect of the A^+ allele on fitness is relatively weak, both selection and genetic drift should be taken into account simultaneously in the calculation. This is an interesting subject, although formulation and analysis of such a system are very difficult and should be the subject of future work. In such an analysis, this model provides a good basis for revealing the evolutionary process of gene transfer from mitochondria to the nucleus.

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